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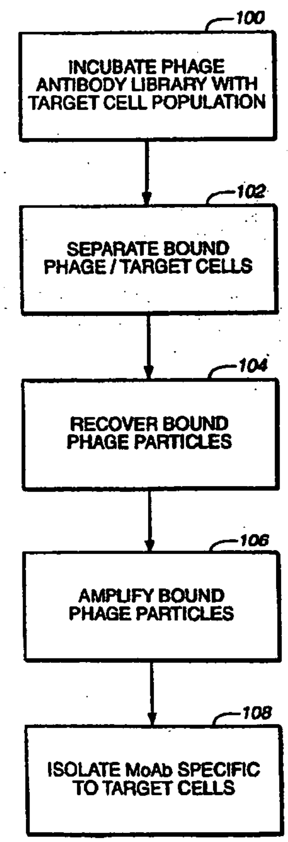
(54) Title: METHOD FOR GENERATING CELL TYPE SPECIFIC PHAGE ANTIBODY LIBRARIES

(57) Abstract

A method for generating monoclonal antibodies directed against previously uncharacterized an unpurified antigens on the surface of target cells in a cell population. The method includes incubating (100) a combinatorial library of antibodies expressed on the surface of filamentous phage particles with a target cell population under conditions sufficient to bind a portion of the phage particles to the target cells. The target cells and bound phage particles are then separated (102) from the unbound phage particles, and the bound phage particles are recovered (104). These phage particles are then amplified (106) to create an enriched library. Monoclonal antibodies specific to the target cell are then isolated (108) from the enriched library for subsequent use.



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Method for Generating Cell Type Specific Phage Antibody Libraries

BACKGROUND OF THE INVENTION

The invention was supported by a grant of the National Institute of Health/National Cancer Institute, Grant No. CA-42551, under which the government may have rights.

Field of the Invention

The invention relates generally to the field of cell type specific phage antibody libraries, and relates specifically to the field of generating such libraries by direct affinity selection on the surface of target cells.

Although the generation of antibody libraries, and more specifically phage antibody libraries, has been done for selected, purified antigens, a method for generating cell type specific phage antibody libraries for identifying, characterizing, and cloning new surface molecules on intact, whole cells has not been developed. Such cell surface molecules are likely to be mediators of important biological functions and may be used in clinical applications as targets for pharmacological intervention and surface markers for identification, purification, and phenotypic dissection of the target cells.

Brief Description of Background Art

The phage antibody technology is a recently developed technique that allows the expression of monoclonal antibodies on the surface of filamentous phage particles (see, for example, Smith, Science 228, 1315-1317 (1985); Barbas et al., Proc. Natl. Acad. Sci. USA 88, 7978-7982 (1991); Clackson et al., Nature 352, 624-628 (1991)).

One advantage of this technology is the linkage of the specificity of a monoclonal antibody with the genetic information encoding the specificity in the same phage particle, thus allowing enrichment of specific phage antibodies by binding to purified antigens attached to a solid phase, followed by elution and amplification of the phage particles. Such a method is described in Kang et al., Proc.Natl.Acad.Sci.USA 88, 4363-4366 (1991). Using micropanning or affinity column techniques, researchers have used this technology to generate antibodies reactive to well-characterized antigens that can be purified in large quantities,

either as natural or recombinant products, including various haptens, tetanus toxoid, HIV and RSV glycoproteins, HBV core and surface antigens, progesterone, thyroglobulin, TNF alpha, CEA, soluble CD4, IL2R p55 chain, lysozyme, bovine serum albumin, trypsin, anhydrotrypsin, and L6 tumor-associated antigen.

However, none of these methods involve using the phage antibody technology to generate antibodies directed against previously unknown and unpurified antigens, including cell surface antigens. Accordingly, there remains a need for a method for rapidly generating monoclonal antibodies directed against unpurified surface antigens on phenotypically defined cell populations using this technology.

SUMMARY OF THE INVENTION

The present invention involves methods for using the phage antibody technology to generate antibodies directed against previously unknown and unpurified antigens on the cell surface, e.g., as a way to discover new antigens on important cell types. Since a phage antibody may be amplified by growing the phage in bacteria, it is possible to recover a single phage particle specifically bound to an antigen in order to generate enough monoclonal antibodies for detailed characterization of the antigen. The high sensitivity of the method enables isolation of antibodies directed against an unpurified antigen in the midst of numerous background antigens.

In the present invention, target antigens on the surface of a phenotypically defined population of cells are used to separate phage antibodies reactive to the antigens from the background. The cell membrane of the target cells can be considered a solid phase support for a set of cell type specific antigens, and the intact target cells can be used as a convenient physical device to separate phage antibodies bound on the surface of the target cells from the unbound phage antibodies.

The inventive method for generating antibodies against previously uncharacterized and unpurified antigens on the surface of target cells in a cell population includes incubating a combinatorial library of antibodies expressed on the surface of filamentous phage particles with a target cell population under conditions sufficient to bind a portion of the phage particles to the target cells. The target cells and bound phage particles are then separated from the unbound phage particles, and the bound phage particles are recovered. These phage particles are then amplified to create an enriched library. Monoclonal antibodies specific to the target cell are then isolated from the enriched library for subsequent use.

To selectively enrich phage antibodies direct against antigens unique to the target cells, the phage antibody library may be preadsorbed with an excess of non-target cells to immunologically remove antibodies directed against antigens present on non-target cells. The adsorption step may be performed after the phage antibody selection and amplification, or prior to the initial incubating step. Alternatively, the removal of these antibodies can be done by subtraction hybridization of DNA from a phage antibody library enriched with the target cells to DNA from a library enriched with non-target cells. The phenotypically defined target cell population may be isolated prior to incubating the target cells with the phage antibody library, or the target cells may be a subpopulation within a heterogeneous cell population and be isolated after incubating with the phage antibody library, thus copurifying the phage antibodies bound to the cell surface antigens.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a flow chart illustrating an embodiment of the present inventive method.

FIGURE 2 is a flow chart illustrating another embodiment of the present inventive method.

FIGURES 3a - 3d are histograms showing indirect immunofluorescence patterns of KG1a cells and mouse thymocytes stained with the unenriched and enriched antibody libraries. The abscissa indicates relative fluorescence intensity, and the ordinate indicates relative cell number.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The process of the present invention includes a method to generate antibodies against cell surface antigens by direct affinity selection of phage antibodies on the surface of target cells. Generally, a phage antibody library is constructed by standard methods and an aliquot of the library is used for incubation with the target cells. Phage antibodies that are specific to and have a high affinity for antigens on the target cells will tightly bind to the cells and can be separated from the rest of the phage antibody library by washing and spinning down the cells. The bound phage particles can then be eluted from the cell surface, recovered, and amplified to yield an enriched phage antibody library.

As illustrated in the flow chart of FIGURE 1, the present method involves a first step of incubating (100) a standard phage antibody library, such as a combinatorial library of antibodies expressed on the surface of filamentous phage

particles, with a target cell population. The cells are incubated under conditions sufficient to bind a portion of the phage particles to the target cells. In the embodiment of FIGURE 1, the target cell population is an isolated cell population. The process of the present invention preferably utilizes a standard combinatorial phage antibody library of 10^7 recombinants, which may be prepared using standard protocols, such as that described in Barbas et al., Methods 2, 119-124 (1991). The cell population may be isolated according to any preselected criteria.

Following incubation (100), the target cells and the phage particles bound thereto are separated (102) from the unbound phage particles. Phage antibodies that are specific to and have a high affinity for antigens on the target cells will tightly bind to the cells and can be separated from the rest of the phage antibody library by centrifugating the cells through a calf serum cushion. Other standard separation methods known by those skilled in the relevant art may be used, examples including but not limited to, cell washing and centrifugation, filtration of the unbound phages through a membrane, and immobilization or capture of the cells on a solid phase. The separated phage particles, or phage antibodies, are then recovered (104). The bound phage particles may be eluted, for example using an acidic buffer, from the cell surface using standard methods. The resulting recovered phage antibodies are amplified (106) to yield an enriched phage antibody library. Repeating this enrichment step several times generates a phage antibody library with high specificity for surface antigens on the target cells. Each round of enrichment increases the representation of antibodies with higher affinity for the target antigens but decreases the diversity of the library. Thus, the amount of enrichment will depend on the particular requirements of the user.

Once the enriched library is generated, monoclonal antibodies specific for the target cells are isolated (108) from the library. Thus, phage antibodies reactive to antigens on the target cell population are obtained. The monoclonal antibodies are isolated (108) using methods known and available to those skilled in the art.

In one form of the invention, direct affinity selection of the phage antibodies on the cell surface is used as described substantially above. In that instance, a population of isolated target cells are incubated with the phage antibody library. The target cells may be in the form of a cell line, or other substantially isolated population of target cells.

In an alternative embodiment, and as illustrated in FIGURE 2, the target cell population is a subpopulation from a heterogeneous cell population before

any isolation. After incubation with the phage antibody library, the target cell population may subsequently be separated (110) from other cells in the heterogeneous population based on phenotypic distinctions, or other appropriate distinctions, thus copurifying the phage particles bound to the cell surface (112).

Preferably, using the copurification (110) method illustrated in FIGURE 2, the target cells are phenotypically defined target cell subpopulations. This allows generation of antibodies against rare but phenotypically defined cell subpopulations that are too few in cell number to allow characterization by conventional methods. In this embodiment of the inventive method, the phage antibody library is first allowed to bind to the total and unpurified cell population. The target subpopulation is then physically separated from the rest of the cells by means of its defining phenotypic characteristics. The phage particles specifically bound to the surface antigens of the target cells are separated (112) out together with the target cell subpopulation.

The separated phage particles can then be recovered (114), amplified (116), and used in another round of enrichment in a manner similar to that described above. Thus, phage antibodies reactive to antigens on the target subpopulation of a heterogeneous population may be selectively enriched by copurification of the target cells with their specific phage antibodies, exploiting the phenotypic characteristics of the target cells. Standard methods of cell separation known to those skilled in the art may be used.

The method to be used for the copurification (112) step may be any of the type known and available to those skilled in the art. Exemplary methods applicable to target cells in suspension include fluorescence-activated cell sorting (FACS), immunological panning, magnetic bead separation, and affinity chromatography. The specific copurification method will depend on availability of materials, the type of target cell subpopulation, and other factors determinable by those skilled in the art.

The cell population used in conjunction with the present method may include whole tissue sections, such as frozen tissue sections, in which the target cell population is identifiable. Exemplary methods for identifying and isolating target cell subpopulations may include use of immunohistochemical markers, physical location of cell populations, or other methods known and used by those skilled in the art. In practicing the present invention using tissue sections, the tissue section is incubated with the phage antibody library under conditions sufficient to bind phage particles to the target cells. The target cells and the bound phage particles are then microdissected from the tissue section, the phage

particles are recovered and amplified, and monoclonal antibodies are isolated in accordance with the method described above.

The preferred embodiment of the invention makes use of the phenotypic characteristics of the target cells to identify phage antibodies of interest by means of their binding to the target cells. In an alternative embodiment, phage antibodies are selected if they are shown to have an identifiable and preselected function, as compared with other phage antibodies without such function.

In that embodiment, phage antibodies may be assayed, for example, for the functional consequences of a phage antibody-target cell binding. One assay may be to measure intracellular calcium concentration. Another effective assay may be used to detect cell activation, cell proliferation, or cell killing, or the blocking of these activities. Yet another assay may be to analyze the expression of preselected genes. Other functional methods of identifying phage antibodies known and available to those skilled in the art may be used.

In practicing the present invention, the resulting phage antibody library may contain antibodies reactive to antigens unique to the target cells and antibodies reactive to antigens present on both the target cells and non-target cells. It may be desirable to selectively remove the antibodies reactive to antigens not unique to the target cells. This may be accomplished by preadsorbing the phage antibody library with an excess of non-target cells to immunologically remove phage antibodies directed against antigens present on non-target cells. This additional step may be performed either prior to incubating the phage antibody library with the cell population, or prior to isolating monoclonal antibodies from the enriched library.

Alternatively, it is possible to preincubate the cell population with a saturating amount of antibodies having known specificity prior to incubating the cell population with the phage antibody library. This immunologically blocks the known antigen binding sites on the target cell surface, and avoids enriching phage antibodies having the known specificity.

The goal of selectively enriching phage antibodies directed against antigens present only on target cells and not on non-target cells can also be achieved by DNA subtraction hybridization. In this method, two cell type specific phage antibody libraries are generated using the present inventive method, one for the target cells and one for the non-target cells. DNA encoding antibodies directed against antigens present on both cell types are then removed from the first library by hybridizing DNA from the first library to an excess of DNA from the second library and subsequently removing the annealed hybrids. The resulting

subtracted phage antibody library will then contain mostly antibodies directed against antigens unique to the target cells. Standard methods of DNA subtraction hybridization known to those skilled in the relevant art, or variations of these methods, may be used in this step.

The invention is further described in the following non-limiting examples.

EXEMPLIFICATION

Example 1

Laboratory mice are immunized against KG1a cells by injection of the cells intraperitonially three times at intervals ranging from six weeks to six months. The KG1a cell line is an available human myeloblastoid cell line, and is characterized in several publications, including Koeffler et al., Blood 56, 265-273 (1980).

Six days following the last injection, spleen cells are isolated from the animals, lymphocytes are separated on a Ficoll-Paque™ gradient, commercially available from Pharmacia LKB Biotechnology, Inc., Piscataway, New Jersey, and total RNA is prepared using standard, known techniques. A combinatorial phage antibody library of 10^7 recombinants is prepared using standard protocols as described in Barbas et al., Methods 2, 119-124 (1991), and as described in further detail below.

KG1a cells are incubated with phage particles from the combinatorial library in binding buffer on ice for 30 minutes, and then spun through a calf serum cushion three times. Phage particles bound to the surface of the cells are eluted in an acid buffer (such as 0.1 M HCl, adjusted with glycine to pH 2.2; and BSA 1mg/ml), recovered, and used to infect XL1-Blue bacteria, available from Stratagene Cloning Systems, La Jolla, California. The phagemids in the infected bacteria are expanded in culture and rescued with helper phage VCSM13 to yield an enriched phage antibody library. The library is then used in another round of enrichment.

This enrichment process is repeated several times, four times in this example. Table 1 lists the number of phage particles recovered after each round of enrichment, showing a steady increase in the number of recovered phage particles after each round. In contrast, a negative control experiment (results not shown) using mouse thymocytes as the target cells for binding with the phage shows no increase in the number of phage particles recovered.

Table 1

Enrichment of phage antibodies by direct affinity selection on the cell surface

	Round 1	Round 2	Round 3	Round 4
No. phage recovered	2×10^5	8×10^5	20×10^5	20×10^5

Antigen binding fragments of the antibodies (Fab fragments) are produced from either the unenriched library or the final library after four rounds of enrichment and tested for reactivity to the KG1a cells by indirect fluorescence-activated cell sorting (FACS) analysis. FIGURES 3(a) through 3(d) are histograms showing fluorescence patterns of KG1a cells and mouse thymocytes stained with the unenriched (a,c) or enriched (b,d) antibody libraries. The abscissa indicates relative fluorescence intensity and the ordinate indicates relative cell number.

The staining of KG1a with the unenriched library (a) is no different from that of the background, whereas staining with the enriched library (b) gives signals approximately twenty times more intense. In contrast, the staining of mouse thymocites with either the unenriched (c) or enriched (d) libraries are both indistinguishable from the background. The KG1a cells and mouse thymocytes have different autofluorescence patterns, thus accounting for the difference in background staining. These results demonstrate that phage antibody libraries can be enriched by the inventive method of direct affinity selection on the cell surface to generate monoclonal antibodies highly reactive to target cells.

To determine the fraction of monoclonal antibodies in the enriched phage antibody library that are specific for KG1a cells, Fab fragments are produced separately from ten individual clones picked at random from the library. FACS analysis of these samples (results not given) shows that the Fab fragments from all of the ten clones strongly react with KG1a cells and with variable patterns, indicating that all of the ten monoclonal antibodies are specific for KG1a and probably recognize at least several different surface antigens on the cells. By extension, the entire enriched antibody library most likely contains a large number of monoclonal antibodies recognizing many different surface antigens on KG1a cells.

Example 2

KG1a cells, which bear the human CD34 antigen on their surface, are stained with CD34-specific antibodies according to Civin et al., J.Immunol 133,

157-165 (1984), and spiked at a ratio of 1 to 10 or 1 to 100 into mouse thymocytes, which are CD34 antigen negative. The cell mixtures are incubated with an unenriched phage antibody library and sorted for CD34 antigen positive (CD34+) cells by FACS. Phage particles bound on the surface of the sorted cells are recovered by acid elution and used to infect XL1-Blue bacteria. An enriched phage antibody library is then produce by helper phage rescue of the phagemids in the infected bacteria and used in another round of enrichment. As shown in Table 2, the number of phage particles recovered from the sorted CD34+ cells increases dramatically after three rounds of selection, suggesting effective enrichment of specific phage antibody by the copurification method described in detail above.

Table 2

Enrichment of phage antibodies by copurification with target cells

(a) KG1a and mouse thymocytes at a ratio of 1:10

	Round 1	Round 2	Round 3
No. CD34+ cells sorted	250,000	250,000	283,000
No. phage recovered	1.5×10^4	4.5×10^4	50×10^4

(b) KG1a and mouse thymocytes at a ratio of 1:100

	Round 1	Round 2	Round 3
No. CD34+ cells sorted	52,000	63,000	80,000
No. phage recovered	0.6×10^4	1.6×10^4	10×10^4

Fab fragments are produced from the enriched library and used for staining KG1a and mouse thymocytes. FACS analysis using these Fab fragments show a strong reaction with the KG1a cells but no reaction with the mouse thymocytes (results not shown). Fab fragments from the unenriched library give signals similar to the background. This result demonstrates the feasibility of producing specific monoclonal antibodies directed against a rare population of cells using the combined strategies of cell surface selection and target cell copurification.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention claimed herein.

All cited literature are incorporated by reference in their entirety.

We claim:

1. Method for generating monoclonal antibodies directed against previously uncharacterized and unpurified antigens on the surface of target cells in a cell population, using a combinatorial library of antibodies expressed on the surface of filamentous phage particles, the method comprising the steps of:

A. incubating the library with the target cells under conditions sufficient to bind a portion of the phage particles to the target cells;

B. separating the target cells and the bound phage particles from unbound phage particles;

C. recovering the phage particles bound to the target cells;

D. amplifying the bound phage particles to create an enriched library; and

E. isolating from the enriched library monoclonal antibodies specific for the target cells.

2. The method of claim 1, wherein the target cells comprise a phenotypically defined target cell subpopulation and the cell population comprises a heterogeneous cell population, the method further comprising, prior to Step A:

purifying the target cell subpopulation from the heterogeneous cell population.

3. The method of claim 1, wherein the target cells comprise a phenotypically defined target cell subpopulation and the cell population comprises a heterogeneous cell population, wherein Step B comprises:

copurifying the target cell subpopulation and the bound phage particles from the heterogeneous cell population and unbound phage particles.

4. The method of claim 3, wherein Step B further comprises:

copurifying the target cells and the bound phage particles by one method selected from the group consisting of: fluorescence-activated cell sorting (FACS); immunological panning; magnetic bead separation; and, affinity chromatography.

5. The method of claim 1, wherein the target cells comprise a defined cell type in a frozen tissue section, and Step B comprises:

identifying the target cells by immunohistochemistry; and

microdissecting the target cells and the bound phage particles from the tissue section.

6. The method of claim 1, wherein the phage particles are phage antibodies, and wherein Step B comprises:

identifying functionally important phage antibodies bound to the target cells by assaying for the consequences of a phage antibody-target cell binding using one assay selected from the group consisting of: measuring intracellular calcium concentration; detecting cell activation, cell proliferation, or cell killing; detecting blocking of cell activation, cell proliferation, or cell killing; and, analyzing expression of preselected genes.

AMENDED CLAIMS

[received by the International Bureau on 24 October 1994 (24.10.94); original claims 6 and 7 replaced by new claim 6 other claims unchanged (3 pages)]

1. Method for generating monoclonal antibodies directed against previously uncharacterized and unpurified antigens on the surface of target cells in a cell population, using a combinatorial library of antibodies expressed on the surface of filamentous phage particles, the method comprising the steps of:

A. incubating the library with the target cells under conditions sufficient to bind a portion of the phage particles to the target cells;

B. separating the target cells and the bound phage particles from unbound phage particles;

C. recovering the phage particles bound to the target cells;

D. amplifying the bound phage particles to create an enriched library; and

E. isolating from the enriched library monoclonal antibodies specific for the target cells.

2. The method of claim 1, wherein the target cells comprise a cell line.

3. The method of claim 1, wherein the target cells comprise a phenotypically defined target cell subpopulation and the cell population comprises a heterogeneous cell population, the method further comprising, prior to Step A:

purifying the target cell subpopulation from the heterogeneous cell population.

4. The method of claim 1, wherein the target cells comprise a phenotypically defined target cell subpopulation and the cell population comprises a heterogeneous cell population, wherein Step B comprises:

copurifying the target cell subpopulation and the bound phage particles from the heterogeneous cell population and unbound phage particles.

5. The method of claim 4, wherein Step B further comprises:

copurifying the target cells and the bound phage particles by one method selected from the group consisting of: fluorescence-activated cell sorting (FACS); immunological panning; magnetic bead separation; and, affinity chromatography.

6. The method of claim 1, wherein the target cells comprise a defined cell type in a frozen tissue section, and Step B comprises:

identifying the target cells by immunohistochemistry; and

microdissecting the target cells and the bound phage particles from the tissue section.

7. The method of claim 1, wherein the phage particles are phage antibodies, and wherein Step B comprises:

identifying functionally important phage antibodies bound to the target cells by assaying for the consequences of a phage antibody-target cell binding using one assay selected from the group consisting of: measuring intracellular calcium concentration; detecting cell activation, cell proliferation, or cell killing; detecting blocking of cell activation, cell proliferation, or cell killing; and, analyzing expression of preselected genes.

8. The method of claim 1, further comprising the step of, prior to Step A:

preadsorbing the phage antibody library with an excess of non-target cells, to immunologically remove phage antibodies directed against antigens present on non-target cells.

9. The method of claim 1, further comprising the step of, prior to Step A:

preincubating the target cells with a saturating amount of preselected antibodies having known affinity in order to block binding sites on the target cells for the preselected antibodies.

10. The method of claim 1, further comprising the step of, prior to Step E:

adsorbing the phage antibody library with an excess of non-target cells to immunologically remove phage antibodies directed against antigens present on non-target cells.

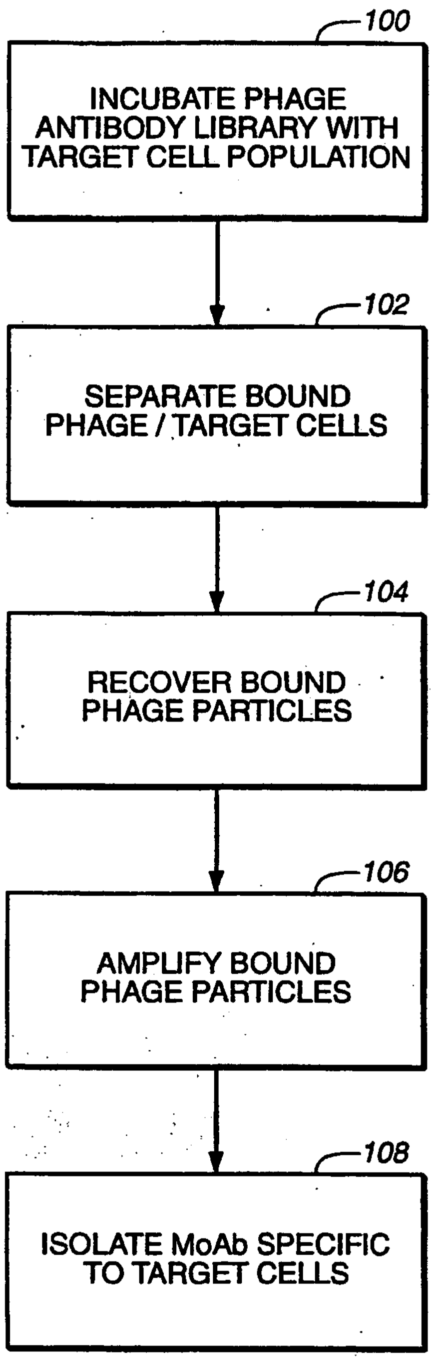
11. The method of claim 1, further comprising the step of, prior to Step E:

removing, from the enriched library, phage antibodies directed against antigens present on non-target cells by DNA subtraction hybridization.

12. The method of claim 1, further comprising, the step of, after Step D, reiterating Step A through Step D to further enrich the library.

13. The cell-type specific phage antibody library produced by the process of claim 1.

14. The monoclonal antibodies generated from the cell type specific phage antibody library produced by the process of claim 1.


100
INCUBATE PHAGE ANTIBODY LIBRARY WITH TARGET CELL POPULATION
102
SEPARATE BOUND PHAGE / TARGET CELLS
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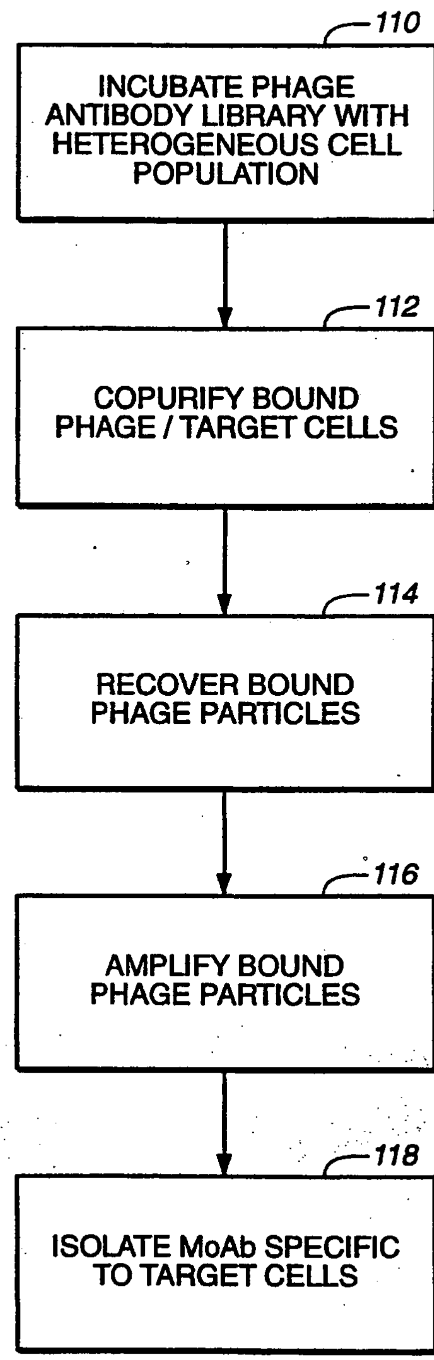
RECOVER BOUND PHAGE PARTICLES
106
AMPLIFY BOUND PHAGE PARTICLES
108
ISOLATE MoAb SPECIFIC TO TARGET CELLS

FIG._1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INCUBATE PHAGE ANTIBODY LIBRARY WITH HETEROGENEOUS CELL POPULATION
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COPURIFY BOUND PHAGE / TARGET CELLS
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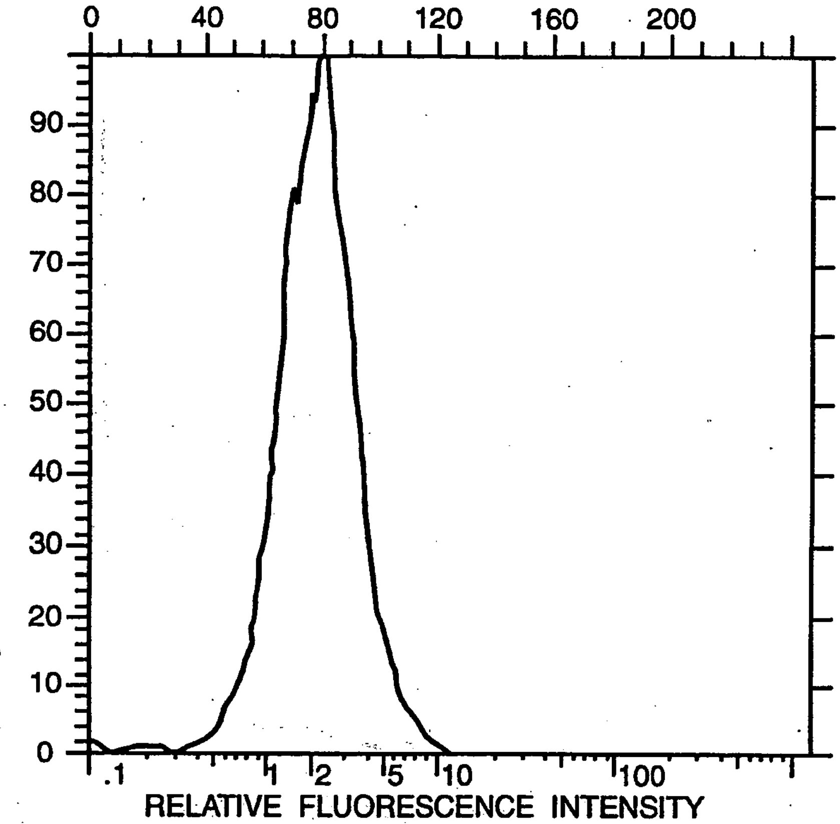
RECOVER BOUND PHAGE PARTICLES
116
AMPLIFY BOUND PHAGE PARTICLES
118
ISOLATE MoAb SPECIFIC TO TARGET CELLS

FIG._2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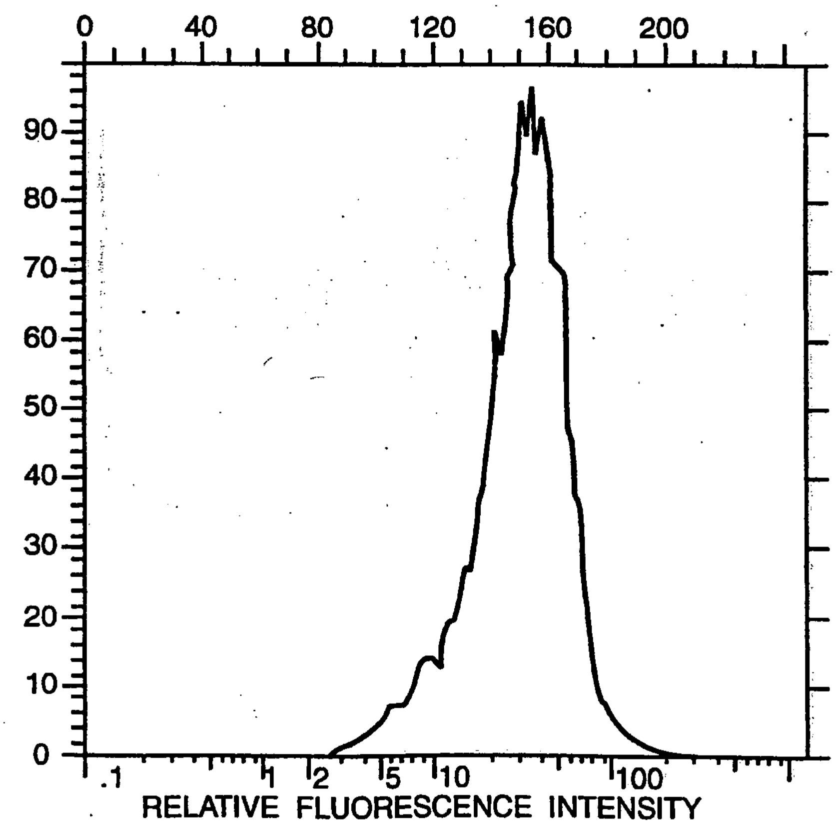
RELATIVE FLUORESCENCE INTENSITY

FIG._3A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.1 1 2 5 10 100
RELATIVE FLUORESCENCE INTENSITY

FIG._3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05124

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 15/28
US CL :530/387.3, 867
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.3, 867

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
SEARCH TERMS: PHAGE AND ANTIBODY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A2, 0,460,607 (HELLSTROM ET AL.) 11 DECEMBER 1991, SEE ENTIRE DOCUMENT	1-6
Y	J. IMMUNOLOGY, VOL. 135, NO. 4, ISSUED OCTOBER 1985, LEDBETTER ET AL., "ANTIBODIES TO TP67 AND TP44 AUGMENT AND SUSTAIN PROLIFERATIVE RESPONSES OF ACTIVATED T CELLS," PAGES 2331-2336, SEE ENTIRE DOCUMENT.	1-6
Y	HARLOW ET AL., "ANTIBODIES A LABORATORY MANUAL" PUBLISHED 1988 BY COLD SPRING HARBOR LABORATORY (N.Y.), PAGES 496-497.	1-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
30 JUNE 1994	28 JUL 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Jill Warden for DONALD E. ADAMS, PH.D. Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

ltional application No.
PCT/US94/05124

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATURE, VOL. 308, ISSUED 08 MARCH 1984, HENDRICK ET AL., "ISOLATION OF CDNA CLONES ENCODING T CELL-SPECIFIC MEMBRANE-ASSOCIATED PROTEINS", PAGES 149-153, SEE ENTIRE DOCUMENT.	1-6